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Exploring the effects of cannabidiol on RAW 264.7 macrophage cell viability and inflammatory cytokine IL-6 production

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May 2020

Abstract

Cannabidiol (CBD) is a non-psychoactive derivative from the marijuana plant. Recently, there has been an explosion of readily available commercial products infused with and marketing CBD contents, and yet there is a paucity of information regarding the specific effects of CBD on immunity. Our laboratory is interested in examining the effects of dietary choices on innate immunity, using the immortalized murine macrophage cell line, RAW 264.7. The objective of this research was twofold. First, to determine if CBD treatments would exert an observable effect on the murine macrophages when cultured in complete media. Therefore, identical experiments were conducted with two culture conditions, complete media versus serum-free media, during CBD treatment. Second, to determine the effects of CBD on the proliferation, activation, and inflammatory cytokine production in lipopolysaccharide (LPS)-activated murine macrophages while also establishing a protocol for treating macrophages with CBD. Macrophage RAW 264.7 cells were incubated with either control, 0.2, or 20 μM of CBD for 24 hours in serum-free media (SFM) or complete media (CM), followed by a 6 hour, 0.01 or 1.0 $\mu\text{g/ml}$ LPS challenge in SFM. Analyses were done on total cells counts, cell viability and proliferation, as well as nitric oxide and pro-inflammatory cytokine interleukin-6 (IL-6) concentrations in cell-conditioned media. We found that macrophages cultured in CM during CBD treatments had higher percentages of live cells compared to those cultured in SFM with the exception of SFM and 0.2 μM CBD treatment, in which percent live cells were comparable to CM percent live counts. Additionally, culturing macrophages in CM, during CBD treatments, resulted in significantly higher cell viability (MTS assay) compared to macrophages in SFM. There was no significant effect of CBD or media type on nitric oxide production. At the highest CBD concentration, (20 μM) in SFM, in addition to the lack of cell growth, proliferation and viability, cells became quiescent and ceased producing inflammatory cytokine IL-6. Our results suggest that with high 20 μM concentrations of CBD, there is a marked effect of decreased cell proliferation and pro-inflammatory IL-6 production.

Keywords: CBD · macrophage · inflammation

Introduction

Cannabidiol Molecule

The marijuana plant, *Cannabis sativa*, has a host of derived molecules and cannabinoids that are of research interest for their various functions and potential health effects. Cannabidiol (CBD) is one such molecule (Figure 1), and is not only the most abundant nonpsychotropic plant cannabinoid, but has been touted as an natural remedy for a variety of health issues such as chronic pain, insomnia and anxiety. Yet another derivative is Δ^9 -tetrahydrocannabinol (THC) which is the major psychoactive ingredient and has also been implicated in various immunomodulatory effects.

Usage of the marijuana plant by people for its fibers, food, rituals, or believed health benefits dates back to pre-Neolithic times (Clarke & Merlin, 2013).

However, twenty years ago, cannabinoids were being researched by few and was of little interest to many researchers. Reports from as early as the 1960s have warned of the potential effects of cannabinoids such as THC and CBD on human health and decreased ability to resist infections (Pertwee, 2005). More recently, there has been an increased growth in the CBD oil market because of factors such as the legalization of cannabis-based products, leading to the incorporation of its oil into our supplements, food, and body care products (MarketWatch, 2019). In the past THC has received considerably more attention than CBD, the recent rise in commercialization and more relaxed regulations have made CBD a topic of interest in healthcare and commercial products alike. Since, CBD's specific effects on the body have only recently started being explored the need for laboratory and clinical research on its specific effects has risen.

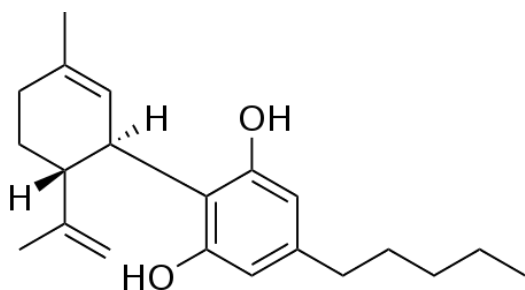


Figure 1: Cannabidiol Molecule

Inflammation

Macrophages are immune cells that scavenge for invading pathogens and help link innate and adaptive immunity by stimulating lymphocytes and other adaptive immune cells to respond to foreign invaders. Macrophages help determine the extent, as well as the type, of immune response that will be mounted against an invading pathogen. Chemokines and cytokines produced by macrophages help recruit and activate other immune cells to the area where the pathogen was encountered. One of the important cytokines produced by macrophages is the pro-inflammatory cytokine interleukin-6 (IL-6) (Janeway *et al.*, 2001). Interleukin-6 is multi-functional, acting as a signaling molecule to hematopoietic stem cells, hepatocytes and immune B cells (Matsuda & Kishimoto, 1998). The importance of IL-6 in many physiological functions makes it the perfect candidate for the initial testing of the immune response to CBD.

Chronic inflammation has been implicated as a cancer causing pathway through tissue destruction and scarring, and reshaping of the tissue architecture as well as contributing to changes in gene expression that may result in tumorigenesis (Hunter, 2012). Understanding how inflammation can be mediated and suppressed in some cases can allow us to curb the onset or even progression of these inflammation-related diseases.

Suppression of the inflammatory response may provide relief for those suffering chronic pain but may be dangerous for lowering the defenses against bacterial and other infections. Normal immune functions is critical for surviving in an environment full

of pathogens and foreign organisms as well as dealing with the internal risk factors such as autoimmune diseases. It is recognized that the age-associated decline in immunologic vigor directly or indirectly contributes to increased susceptibility to infectious diseases, meaning avoiding decreasing the inflammatory response to a drastic extent may prove deadly (Wu *et al.*, 2008). Determining how inflammatory function is affected by various factors, such as CBD and CBD concentration, are important to understand in order to decide how it would be used commercially and in the healthcare setting.

Effects of Cannabidiol on Inflammation

With the potential benefits of CBD, there have been studies done on various cell lines and mouse models to test its immunomodulating effects. The proposed immunomodulating effects of CBD are thought to be related to its strong immunosuppressive effects due to its ability to suppress the proliferation of lymphocytes and production of inflammatory proteins in mice treated with high doses (Kaplan *et al.*, 2008).

The way in which the CBD molecule is able to have an immunomodulating effect on cells within an organism is through the CB1 and CB2 receptors. It has been discovered that the CB2 receptor is mainly expressed in immune cells including monocytes, the precursors to macrophages. Additionally, it has been found that in mouse monocytes that a predominance of CB2 receptors resulted in a stronger anti-inflammatory response, such as an inhibition of chemotactic movement in response to monocyte chemo-attractant protein 1 when stimulated by cannabinoids (Han *et al.*, 2009). Essentially, CBD acts as an inverse antagonist at the CB2 receptors meaning that it is able to inhibit the migration of immune cells therefore exerting an anti-inflammatory effect (Watt & Karl, 2017).

With the increase in legalization and interest in the CBD molecule, it has been extensively studied in the last decade. There is a current trend in the medical industry to include CBD as a possible treatment for many ailments, from neurologic disorders like epilepsy and seizures to inflammatory and autoimmune diseases. There is current research on the

beneficial effects of CBD in animal models of multiple sclerosis, rheumatoid arthritis, diabetes, and Alzheimer’s disease, among others (Kozela *et al.*, 2011; Malfait *et al.*, 2000; Weiss *et al.*, 2008; Watt and Karl, 2017).

The benefit of decreased anti-inflammatory proteins released from macrophages has also been tested in diabetic mouse models. In young non-obese diabetes-prone mice administered with CBD, symptoms of initial or latent diabetes stages were ameliorated and levels of pro-inflammatory cytokine IL-12 produced by splenocytes were significantly reduced (Weiss *et al.*, 2008). Although there is plenty of scientific literature using animal or cell-based immunological models for CBD research, the investigations into the effects of CBD therapy on the immunity of human subjects is quite limited. Past studies with human subjects have primarily focused on healthy individuals, rather than those suffering from chronic disease, and the use of cannabis on circulating pro-inflammatory cytokine concentrations in the blood (National Academies of Sciences, Engineering, and Medicine, 2017). With the continued research into the effects of CBD in immunity and positive therapeutic results, future directions may include more human based studies.

Research Objectives

Culture Conditions The primary objective of this research was to determine the appropriate methodology and protocol for treating murine macrophages with physiological concentrations of CBD *in vitro*. Since we did not know if CBD would only be taken up by serum-starved cells we designed the experiments to test complete versus serum-free media.

Effects on Inflammation If CBD can suppress inflammatory molecule production in murine models then it makes sense to research its immunomodulating ability in mouse macrophages. The important question we ask is: do physiological doses of CBD affect inflammatory parameters in mouse macrophages? Therefore the secondary purpose

was to test the effects of CBD on the proliferation, activation, and inflammatory cytokine production in lipopolysaccharide (LPS)-activated murine macrophages.

Experimental Design

To test our first objective of creating a protocol to determine if CBD would have an observed effect in complete media we created two distinct culture conditions using complete media or serum-free media for the duration of the CBD treatments. Serum-free media lacks bovine serum albumin compared to complete media. The activation of macrophages via LPS was still done in SFM as the macrophage activation efficacy of LPS is diminished in complete media.

In addition to the culturing of macrophages within two different media types, the quality and quantity of cells were analyzed as well as important inflammatory markers, nitric oxide and pro-inflammatory cytokine IL-6, were quantified from the conditioned media after LPS-stimulation. This was necessary in order to address our secondary objective: measuring the concentration of the pro-inflammatory molecule IL-6 to observe the potential immunomodulating effects of varying CBD concentrations on mouse macrophage immune cells.

Materials and Methods

Materials

Murine macrophages (RAW 264.7) were purchased from ATCC (Manassass, VA). Dulbecco’s modified Eagle’s medium (DMEM), phosphate buffered saline (PBS), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Fisher Scientific (Pittsburg, PA). The cannabidiol molecule was obtained from Cayman (item: 90081) suspended in methanol. The methanol was replaced with ethanol by drying out the methanol with N₂ gas and reconstituting the CBD in 2 ml of pure ethanol. Lipopolysaccharide for macrophage stimulation was obtained from *E. coli*. The interleukin IL-6 enzyme-linked immunosorbent assays (ELISA) were purchased from

R&D systems (Minneapolis, MN). Griess assay's for nitric oxide (NO) quantification and MTS assay for cell proliferation quantification were obtained from Promega (Madison, Wisconsin).

Cell Culture and Treatment

RAW 264.7 murine macrophages were cultured in Gibco DMEM with 10% FBS and 1% penicillin-streptomycin in polystyrene 6-well plates. Cells were incubated at 37 °C in a humidified environment of 95% O₂ 5% CO₂. Complete and serum-free media were prepared the same with antibiotics for incubation of macrophages, with the exception of SFM which lacked bovine serum albumin compared to complete media. Medium was changed every two days and the cells were not grown beyond 80% confluency during expansion. Experiments were performed with cells not surpassing 15 passages. For CBD treatments, cells were grown until 80-90% confluence followed by a 24 hour CBD treatment, either an ethanol control, 0.2 µg/ml, or 20 µg/ml of CBD. Following CBD treatment, supernatant was removed, cells were washed twice with 1X PBS and serum-free media was added along with 0.01 or 0.1 µg/ml of LPS. After a 6 hr incubation, cell-conditioned media was collected and stored in -80°C until NO, and IL-6 analysis. Cell counts were performed immediately following the 6 hr LPS challenge. Experiments were conducted five times.

Cell Proliferation Analysis

An MTS assay was run concurrently with each experiment. Cells were seeded in a 96-well plate at an initial concentration of 0.01×10^6 cells per well. Once a confluency of 80% was reached, macrophages were treated with appropriate CBD concentrations of either control, 0.2 µg/ml, or 20 µg/ml for 24 hrs. Subsequently, after 6 hr LPS stimulation, CellTiter 96® AQueous One Solution Reagent was added directly to wells and incubated at 37 °C. Measurements were taken at 1 and 2 hrs by Epoch spectrophotometer at 490 nm.

Cytotoxicity Analysis

During collection of cell conditioned media, 1 ml was retained in the wells for cell scraping and cell counts. From each well 100 µl of cells and 100 µl of trypan blue were mixed. From this mixture, 10µl were applied to hemocytometer slides and inserted into the Countess™ II FL cell counter (Fisher Scientific, Pittsburgh, PA), to determine total concentration of cells, percent live, and dead cells.

Nitric Oxide (NO) Concentration

Analysis of NO concentration in samples was done using a Griess Reagent kit, with samples tested in triplicate. The standard curve was used to extrapolate the concentrations of NO from the samples. Measurements were taken by Epoch spectrophotometer at 548 nm.

IL-6 Concentration

Analysis of IL-6 in samples was done using enzyme-linked immunosorbent assay (ELISA). Plates were coated with capture antibody 24 hr before use with samples tested in triplicate. The standard curve was used to extrapolate the concentrations of IL-6 from the samples.

Statistics

Results are expressed as a mean \pm SEM. Nitric oxide and percent live cells data were normalized using a log base 10 transformation, and ArcSin square root, respectively. Concentrations of IL-6 and NO in cell culture medium and were analyzed using Multiway split-plot ANOVA. When treatment effects were detected ($p < 0.05$), means were separated using Tukey's HSD. For the IL-6, NO, percent live, MTS, and total cells, the sources of variation included experiment, treatment, experiment*treatment, and well(experiment*treatment), with the experiment considered a random variable. All statistical analyses were performed using JMP Pro 14 while figures and images were created via Tableau.

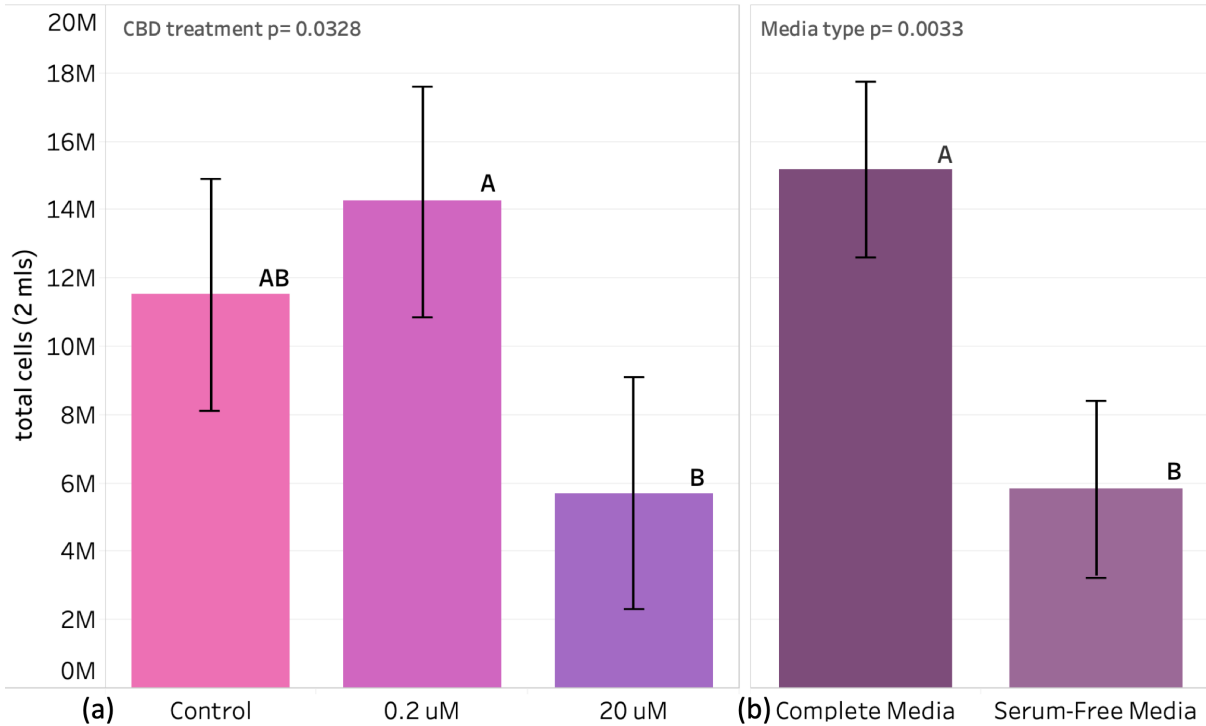


Figure 2: Total cells measured by cell count of RAW 264.7 cells treated with 1.0 $\mu\text{g/ml}$ LPS after CBD pre-incubation (a) Average total number of cells counted in both serum-free and complete media and separated by CBD treatment ($p=0.03$). (b) Average total number of cells counted separated by media type ($p=0.003$). Treatments were compared using a split-plot ANOVA, followed by post-hoc Tukey-Kramer HSD. Significant differences are represented with different letters ($p < 0.05$). Bars represent the mean \pm pooled S.E.M. ($n = 5$).

Results

In our study, we evaluated the importance of complete versus serum-free media in examining the effects of CBD on various cytokines and cellular functions in *in vitro* LPS-stimulated murine macrophage RAW cells. In order to determine if maintaining the murine macrophages in serum-free media during CBD incubation would have an effect on cell viability RAW 264.7 cells were incubated with either control, 0.2, or 20 μM of CBD for 24 hour in serum-free media (SFM) or complete media (CM), followed by a 6 hour, 0.01 or 1.0 $\mu\text{g/ml}$ LPS challenge in SFM. These results suggest that concentrations of 0.2 and 20 μM CBD have significant effects on murine macrophage proliferation (Figure 3), viability (Figure 2 & 4), and IL-6

production (Figure 6) in both complete media and serum-free media.

Cell Viability

Amongst the CBD treatments, for both media types, total cells counts were significantly affected ($p = 0.033$) by the 0.2 μM CBD treatment averaging higher in cell counts than 20 μM CBD treatments (12465375 ± 2784496 , 4944137 ± 2784496) (Figure 2a). However, both 0.2 and 20 μM CBD treatments were not significantly different than the control containing no CBD.

When comparing total cells by media type treatment, cells grown in complete media during CBD treatment incubation resulted in a sig-

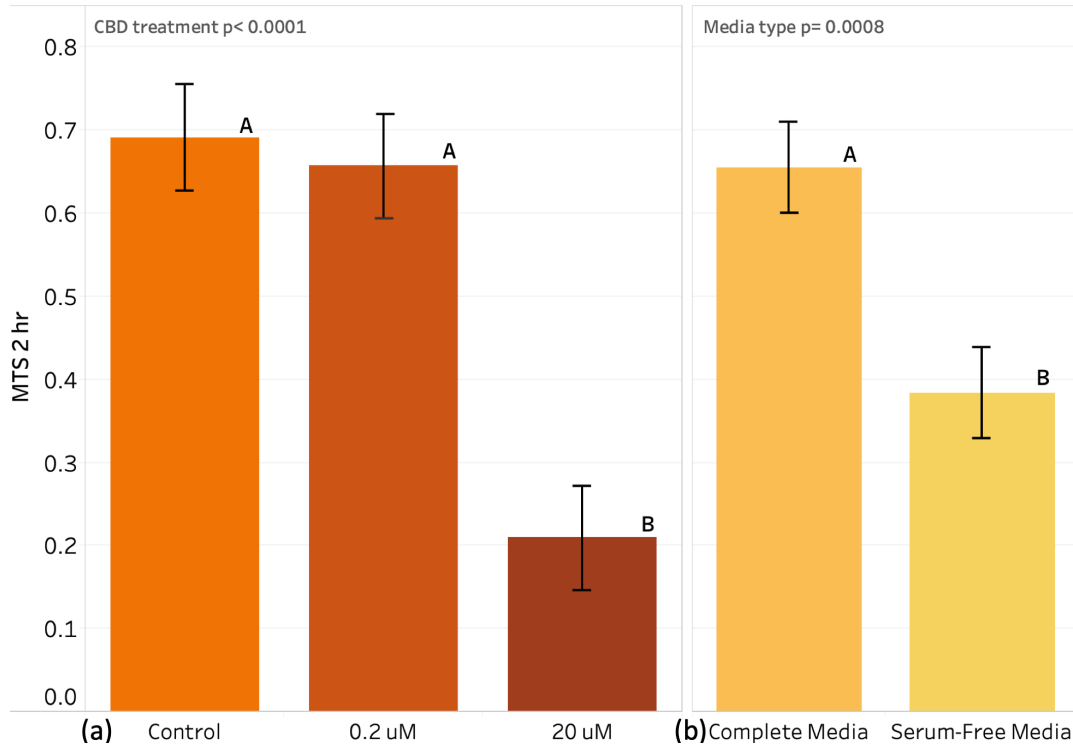


Figure 3: Cell proliferation measured by MTS of RAW 264.7 cells activated with LPS after CBD 24 hr pre-incubation (a) Average cell proliferation measured in both media types for different CBD treatments ($p < 0.0001$) (b) Calculated average cell proliferation separated by media type ($p=0.0008$). Treatments were compared using a split-plot ANOVA, followed by post-hoc Tukey-Kramer HSD. Significant differences are represented with different letters ($p < 0.05$). Bars represent the mean \pm pooled S.E.M. ($n = 4$).

nificantly higher average cell count (12498542 ± 2556384 , 5592883 ± 2556384 ; $p = 0.003$) than its serum-free treatment counter part (Figure 2b). CBD concentrations had similar effects on cell viability regardless of media type on cell viability (CM control 13221750 ± 3377627 , $0.2 \mu\text{M}$ 14805750 ± 3377627 , $20 \mu\text{M}$ 13221750 ± 3377627 and SFM control 6233500 ± 3377627 , $0.2 \mu\text{M}$ 10125000 ± 3377627 , $20 \mu\text{M}$ 420150 ± 3377627 total cells; $p = 0.73$).

Cell Proliferation

Cell proliferation was determined via MTS proliferation assay. Murine macrophages in both media types treated with $20 \mu\text{M}$ CBD on average had significantly lower proliferation than control or $0.2 \mu\text{M}$ treatments

(0.2 ± 0.06 , 0.69 ± 0.06 , 0.65 ± 0.06 ; $p < 0.0001$) (Figure 3a).

When comparing the two media types, serum-free media had an average proliferation of cells much lower than cells cultured in complete media during CBD treatment (0.38 ± 0.53 and 0.65 ± 0.53 ; $p = 0.0008$) (Figure 3b). There was no significant effect of a media type and CBD treatment interaction on cell proliferation ($p = 0.09$).

Cytotoxicity Analysis

Cytotoxicity of the CBD treatments and media types was tested by using the Trypan blue exclusion method to measure the percentage of murine macrophage live cells (Figure 4). There was an in-

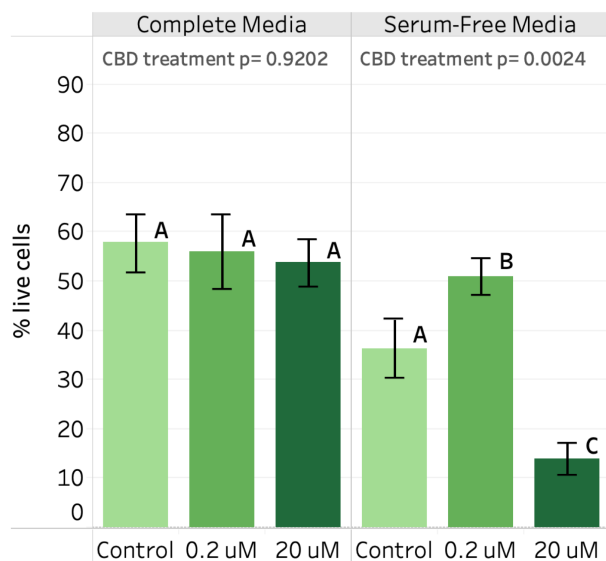


Figure 4: Percent live RAW 264.7 cells after 6 hr LPS challenge with CBD 24 hr pre-incubation. Average percentage of live cells divided by different media types and CBD treatments. Complete media $p = 0.92$ while serum-free media $p = 0.002$. Data was normalized using a $\sqrt{\arcsin}$ transformation and treatments were compared using a split-plot ANOVA, followed by post-hoc Tukey-Kramer HSD. Significant differences are represented with different letters ($p < 0.05$). Bars represent the mean \pm individual S.E.M. ($n = 5$).

teraction effect between media type and CBD treatment ($p = 0.0053$) which was further analyzed by media type to reveal that macrophages cultured in complete media had no difference in percent live cells across all CBD treatments ($p = 0.92$).

Meanwhile, cells grown in serum-free media had significantly varied percent live counts based on CBD treatment with the 0.2 μM CBD treatment resulting in much higher percent live cells than the control and 20 μM CBD treatment ($p = 0.0024$). Overall, SFM+20 μM CBD treatment resulted in the lowest percent live count. Macrophages cultured in complete media had higher percentages of live cells compared to those cultured in serum-free media ($53.2 \pm 5.6\%$ vs $35.2 \pm 5.6\%$ live; $p < 0.0001$).

There was no significant effect of LPS concentration (0.1 versus 1.0 $\mu\text{g/ml}$) for stimulation ($p = 0.45$)

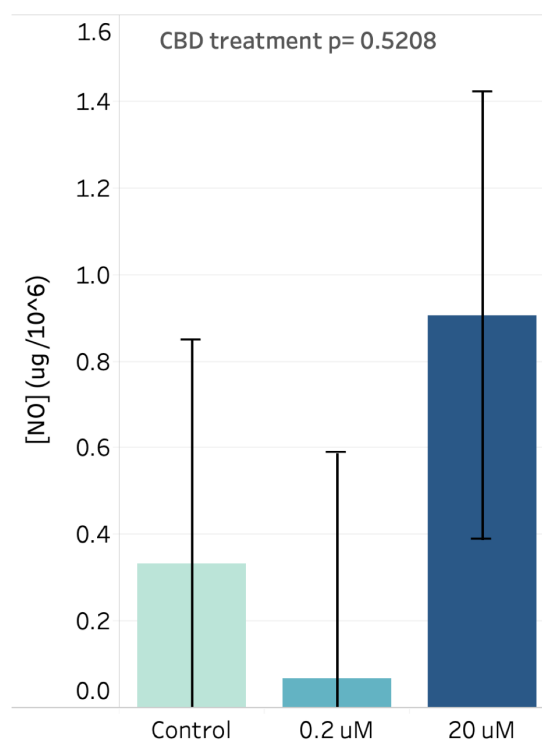


Figure 5: Nitric oxide production by RAW 264.7 cells treated 1.0 $\mu\text{g/ml}$ with LPS after CBD 24 hr pre-incubation. Calculated average of nitric oxide production for both complete and serum-free media and separated by CBD concentration ($n = 4$). Data was normalized using a log transformation and treatments were compared using a split-plot ANOVA. Bars represent the mean \pm pooled S.E.M ($p < 0.05$).

on test effects of CBD or media type.

Nitric Oxide (NO) Concentration

Nitric oxide levels were quantified in order to examine the activation level of the macrophages (Figure 5). There was no significant difference in NO production from the RAW macrophages between CBD treatment concentrations or media type ($p = 0.52$). On average, amongst the three CBD treatment concentrations, cell production of nitric oxide averaged 0.55 ± 0.52 .

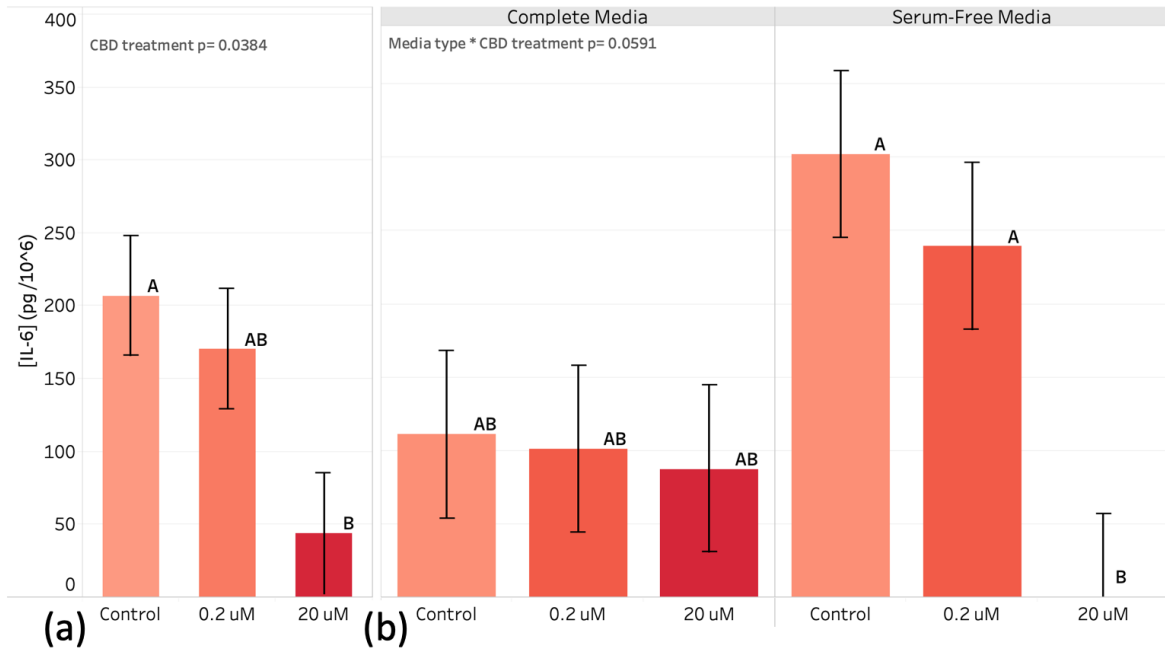


Figure 6: IL-6 concentrations in conditioned media produced by RAW 264.7 cells treated with 1.0 $\mu\text{g}/\text{ml}$ LPS after CBD pre-incubation (a) Average IL-6 produced calculated from both media types and separated by CBD concentration ($p = 0.038$). (b) Average IL-6 produced with bars separated by CBD concentration within different media types ($p = 0.059$). Treatments were compared using a split-plot ANOVA, followed by post-hoc Tukey-Kramer HSD. Significant differences are represented with different letters ($p < 0.05$). Bars represent the mean \pm pooled S.E.M ($n = 2$).

IL-6 Concentration

Only experiments that stimulated macrophages using 1.0 $\mu\text{g}/\text{ml}$ of LPS after CBD treatment were analyzed for IL-6 production levels ($n=2$). On average, between the two media types, the 20 μM CBD treatment had significantly decreased cellular IL-6 production compared to the control (43.7 ± 41.43 and $206.8 \pm 41.7\text{pg}/10^6$, $p = 0.038$, Figure 6a).

When CBD treatment effects were separated based on media type, there was a trend that indicated that in serum-free media, 20 μM CBD treatment resulted in a complete elimination of IL-6 production compared to control and 0.2 μM CBD treatments ($7.1 \times 10^{-14} \pm 57.4$, 302.3 ± 57.4 , $239.6 \pm 57.4\text{pg}/10^6$; $p = 0.059$, Figure 6b). Meanwhile, in complete-media and CBD treated cells, there was no difference between the IL-6 concentrations produced across all of the

CBD treatments. Overall, there was no significant effect of media type on cell IL-6 production ($p = 0.09$).

Discussion

Methodology

While drawing on past research into CBD's effects on other cell lines and ailments, our project aimed to establish a protocol for the culturing and treatment of murine macrophages with CBD while observing the effects of varying concentrations of CBD on cell viability, proliferation, cytotoxicity, NO and IL-6 production. This methodology will hopefully enable further research into CBD's effects on murine macrophages while also providing a useful tool for analysis of additional specific cytokines that affect

inflammation.

Based on the results of this study we would proceed with the use of complete media for future experiments testing the effects of CBD on murine macrophages. In our previous work with fatty acids and murine macrophages we used serum-free media as a means of ensuring the delivery of our fatty acid treatment into the cells. To determine if this was necessary for CBD we chose to duplicate the CBD treatments into two media type conditions, serum-free and complete media. There was an observed effect of CBD on macrophage function compared to the control in experiments with the complete media condition. Therefore it can be determined that cannabidiol has an effect on macrophages with or without complete media. It is still necessary to utilize serum-free media when conducting the macrophage LPS challenge post CBD treatment as LPS is deactivated when used with complete media.

Using complete media is favorable as on average, experiments result in higher total cells, percent live counts, and proliferation than those with serum-free media. Cell proliferation was measured to have the same effects for the CBD treatments regardless of media-type, allowing us to choose the media type based on the total cell and percent live differences. However, within our IL-6 results, differences between CBD concentrations are more easily observed in the SFM. In previous research using complete media and CBD pre-treatments on murine macrophages, Rajan *et al.* (2016) performed 2 hour 5 μ M CBD treatments followed by 24 hr LPS stimulation. A longer LPS stimulation may be necessary for future complete media CBD treatment experiments to see more observable differences between cytokine production levels. Based on our results of this study it can be expected that future CBD experiments using complete media should result in similar percent live values across different CBD treatments in the 0.2 to 20 μ M concentration range. Additionally, cells grown in serum-free media during the CBD pre-treatment period did not grow as much as those grown in complete media during this period which proves difficult to measure cytokine levels when there are minimal cells producing them.

CBD effects

These results suggest that in general, concentrations of 0.2 and 20 μ M CBD have no detrimental effects on murine macrophage viability. We are unable to conclude on the result of the nitric oxide assay as there were no significant effects due to large variability of measured nitric oxide concentrations for each treatment and media type.

We are able to see that for percent live cells in complete media, there is no evident cytotoxic effect of the higher concentration of 20 μ M CBD. With the serum-free media there is significantly lower percent live cells but this is also in combination with a general lack of total cells and significantly lower proliferation levels (MTS assay) signifying a lack of growth of cells and cell death. It is known that cannabinoid receptors modulate several signalling pathways that are directly involved in the control of cell proliferation and survival. For example, various cannabinoids have been found to exert an antiproliferative action on a wide spectrum of cultured tumor cells (Pertwee, 2005). With this lack of cells in the SFM+20 μ M CBD treatment, it is understandable why IL-6 production is decreased to almost zero. Therefore, the 20 μ M CBD concentration is not more cytotoxic than 0.2 μ M CBD concentrations but, for the higher concentration there is a decrease in proliferation as well as a decrease in the inflammatory cytokine IL-6. These effects can be translated to positive results for certain ailments, for it has been observed that in pre-diabetic mice treated with CBD, there was a significant decrease of IL-6 in mouse plasma, as well as a decrease in risk of diabetes development in adulthood (Weiss *et al.*, 2009).

When comparing total cell with MTS proliferation data, there is a pattern in the effect of CBD concentration on the average values, with the exception of a slight increase in 0.2 μ M CBD treated total cells counts compared to the MTS data which has lower proliferation for the same treatment. At the high 20 μ M CBD concentration, cells became quiescent and stopped producing inflammatory cytokine IL-6 in addition to the lack of cell growth, proliferation and viability. In encephalitogenic MOG-specific T cells treated with CBD, there was an antiproliferatory ef-

fect in antigen-induced cell proliferation (Kozela et al., 2011). These RAW macrophages were also stimulated via LPS antigens after CBD treatment and display a similar inhibitory effect on proliferation, especially with increased concentrations of 20 μ M CBD.

It may be possible to increase the effectiveness of the CBD molecule by altering its binding ability to the CB1 and CB2 receptors. An experiment was conducted in which four derivatives of CBD were tested for their ability to modulate NO, TNF- α , reactive oxygen intermediates and their binding to CB1. Two of the derivatives showed an increased suppressive effect of these inflammatory markers which may be a path for developing therapeutic agents to modulate nitric oxide and TNF- α production (Ben-Shabbat *et al.*, 2006).

Conclusion

The study of the effects of the cannabidiol molecule are a quickly developing field and the need for understanding how to culture cells with this molecule is the most basic of information needed to proceed. We have been able to determine a methodology for the culture and treatment of murine macrophages with different concentrations of CBD. The establishment of this methodology of using complete media has provided us with the groundwork for our initial findings into the variable effects of different CBD concentrations on the functions and cytokine production of murine macrophages.

Future Directions

With this exploration we have identified the effects of CBD on a few specific cellular markers, and the pro-inflammatory cytokine IL-6. Nevertheless, there are many other cytokines that macrophages produced that impact inflammatory as well as other important biological pathways. By using the protocol we established for culturing macrophages with CBD we can now examine these other cytokines. One such macrophage product that is of interest to us is TNF- α because suppression of it inhibits inflammatory damage. Additionally, the cannabinoid receptor CB2

found mainly on immune cells has a role in modulating cytokine release and is a target of interest for us (Pertwee, 2005). Finally, we have raised the question: do physiological doses of CBD affect inflammatory parameters in mouse macrophages and adipocytes? We will work to observe inflammatory molecule production by adipocytes and macrophages, separately, as well as co-incubated after treatment with CBD.

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